Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims.

Listing of Claims:

1. (Currently Amended) A method for determining the effect of a test agent on an engineered cartilage tissue~~, a tissue engineered cartilage matrix, or the cells thereof~~ comprising:

(A) culturing an engineered cartilage tissue comprising the steps of:

(i) culturing isolated chondrogenic cells for an amount of time effective for allowing formation of a chondrogenic cell-associated matrix ~~but short enough such that the collagen fibrils in the cell-associated matrix do not become overly crosslinked, wherein the matrix loses roughly half of its proteoglycan content within 24 hours after treatment with IL-1 and loss of the proteoglycan content can be measured without the use of a radioactive agent~~; and

(ii) culturing the chondrogenic cells with the cell-associated matrix on a semipermeable membrane in the presence of a growth factor for a time effective for allowing formation of the engineered cartilage tissue;

(B) contacting one or more test agents with one or more cells or tissues selected from the group consisting of (a) the isolated chondrogenic cells prior to (i), (b) the chondrogenic cells during (i), (c) the chondrogenic cells and cell-associated matrix prior to (ii), (d) the chondrogenic cells and cell-associated matrix during (ii), and (e) the engineered cartilage tissue; and

(C) measuring proteoglycan loss from the ~~effect, if any, the one or more test agents has on the contacted cells, matrix or~~ engineered cartilage tissue at a time not more than 12 days from culturing, without use of a radioactive agent, ~~whereby a change in the physical properties or chemical composition of the contacted cells, matrix or tissue relative to a control indicates that~~ to determine whether the test agent has an effect on the contacted cells or tissue.

2. (Original) The method of claim 1 wherein the chondrogenic cell-associated matrix comprises aggrecan, collagen types II, IX and XI, matrix proteins and hyaluronan.

3. (Original) The method of claim 1 wherein the engineered cartilage tissue comprises collagen types II, IX and XI, hyaluronan and at least about 5 mg/cc^3 aggrecan, wherein the ratio of aggrecan to hyaluronan is about 10:1 to about 200:1, and the ratio of aggrecan to collagen is about 1:1 to about 10:1.

4. (Original) The method of claim 1 wherein the isolated chondrogenic cells are from articular cartilage.

5. (Original) The method of claim 1 wherein the isolated chondrogenic cells are from costal cartilage, nasal cartilage, auricular cartilage, tracheal cartilage, epiglottic cartilage, thyroid cartilage, arytenoid cartilage or cricoid cartilage.

6. (Original) The method of claim 1 wherein the isolated chondrogenic cells are from fibrocartilage.

7. (Original) The method of claim 6 wherein the fibrocartilage is ligament, tendon, meniscus or intervertebral disc.

8. (Original) The method of claim 1 wherein step (i) comprises culturing the chondrogenic cells on an alginate medium.

9.-10. (Cancelled).

11. (Previously Presented) The method of claim 10 wherein step (C) further comprises enzymatically degrading the engineered cartilage tissue.

12. (Original) The method of claim 11 wherein step (C) further comprises staining the enzymatically degraded engineered cartilage tissue with a dye.

13. (Original) The method of claim 1 wherein the engineered cartilage tissue is removed from the semipermeable membrane prior to being contacted with the test agent.

14. (Original) The method of claim 1 further comprising:

(D) identifying one or more test agents that have desirable properties; and

(E) producing the one or more test agents as a therapeutic drug.

15. (Withdrawn) A kit for determining the effect of a test agent on a tissue engineered cartilage matrix comprising instructions for carrying out the method of claim 1.

16. (Withdrawn) The kit of claim 15 further comprising one or more of:

(i) one or more reagents;

(ii) an enzyme capable of degrading the engineered cartilage tissue;

(iii) a dye capable of labeling a component of the engineered cartilage tissue; and

(iv) an antibody capable of labeling a component of the engineered cartilage tissue.

17. (Currently Amended) A method for determining the effect of a test agent on an engineered cartilage tissue~~, a tissue engineered cartilage matrix, or the cells thereof~~ comprising:

(A) culturing an engineered cartilage tissue comprising the steps of:

(i) culturing isolated chondrogenic cells for an amount of time effective for allowing formation of a chondrogenic cell-associated matrix ~~but short enough such that the collagen fibrils in the cell-associated matrix do not become overly crosslinked, wherein the matrix loses roughly half of its proteoglycan content within 24 hours after treatment with IL-1 and loss of the proteoglycan content can be measured without the use of a radioactive agent~~; and

(ii) culturing the chondrogenic cells with the cell-associated matrix on a semipermeable membrane in the presence of a growth factor for a time effective for allowing formation of the engineered cartilage tissue;

(B) contacting one or more test agents with one or more cells or tissues selected from the group consisting of (a) the isolated chondrogenic cells prior to (i), (b) the chondrogenic cells during (i), (c) the chondrogenic cells and cell-associated matrix prior to (ii), (d) the chondrogenic cells and cell-associated matrix during (ii), and (e) the engineered cartilage tissue in the presence of a known modulator of cartilage tissue; and

(C) measuring proteoglycan loss from the ~~effect, if any, the one or more test agents has on the contacted cells, matrix or~~ engineered cartilage tissue at a time not more than 12 days from culturing, without use of a radioactive agent, ~~whereby a change in the physical properties or chemical composition of the contacted cells, matrix or tissue relative to a control indicates that~~ to determine whether the test agent has an effect on the contacted cells or tissue.

18. (Original) The method of claim 17 wherein the chondrogenic cell-associated matrix comprises aggrecan, collagen types II, IX and XI, and hyaluronan.

19. (Original) The method of claim 17 wherein the isolated chondrogenic cells are from articular cartilage.

20. (Original) The method of claim 17 wherein the isolated chondrogenic cells are from costal cartilage, nasal cartilage, auricular cartilage, tracheal cartilage, epiglottic cartilage, thyroid cartilage, arytenoid cartilage or cricoid cartilage.

21. (Original) The method of claim 17 wherein the isolated chondrogenic cells are from fibrocartilage.

22. (Original) The method of claim 21 wherein the fibrocartilage is ligament, tendon, meniscus or intervertebral disc.

23. (Original) The method of claim 17 wherein step (i) comprises culturing the chondrogenic cells on an alginate medium.

24. (Original) The method of claim 17 wherein the engineered cartilage tissue comprises collagen types II, IX and XI, hyaluronan and at least about 5 mg/cc^3 aggrecan, wherein the ratio of aggrecan to hyaluronan is about 10:1 to about 200:1, and the ratio of aggrecan to collagen is about 1:1 to about 10:1.

25.-26. (Cancelled).

27. (Previously Presented) The method of claim 26 wherein step (C) further comprises enzymatically degrading the engineered cartilage tissue.

28. (Original) The method of claim 27 wherein step (C) further comprises staining the enzymatically degraded engineered cartilage tissue with a dye.

29. (Original) The method of claim 17 wherein the modulator of the engineered cartilage tissue is a matrix stimulating agent, cytokine or TNF-α.

30. (Original) The method of claim 29 wherein the cytokine is interleukin-1.

31. (Withdrawn) A kit for determining the effect of a test agent on an engineered cartilage tissue comprising instructions for carrying out the method of claim 17.

32. (Withdrawn) The kit of claim 31 further comprising one or more of:

(i) one or more reagents;

(ii) an enzyme capable of degrading the engineered cartilage tissue;

(iii) a dye capable of labeling a component of the engineered cartilage tissue; and

(iv) an antibody capable of detecting a component of the engineered cartilage tissue.

33. (Original) The method of claim 17 further comprising:

(D) identifying one or more test agents that have desirable properties; and

(E) producing the one or more test agents as a therapeutic drug.

34. (Original) The method of claim 17 further comprising removing the engineered cartilage tissue from the semipermeable membrane prior to contacting the engineered cartilage tissue with the test agent.

35. (Original) The method of claim 17 wherein steps (A) and (B) occur in the same well of a multiwell plate.